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Epitope-Based Peptide Vaccine Design Against Mokola Rabies Virus Glycoprotein G Utilizing In Silico Approaches

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Abstract

Background: *Lyssavirus* is considered as a neglected, zoonotic and tropical virus. Among all the *Lyssavirus* species known to exist today, Mokola virus is unique and appears to be exclusive to Africa. This virus is responsible for a meningoencephalomyelitis in mammals therefore; in silico prediction of epitopes of appropriate protein residues is important to produce a peptide vaccine with powerful immunogenic and minimal allergic effect. The aim of this study was to design a vaccine for Mokola virus using its glycoprotein peptides as an immunogen to stimulate protective immune response.

Methods and materials: Glycoprotein G Sequences of Mokola was explored from NCBI then the sequences were aligned to obtain conserved regions. The nominees epitopes from Immune Epitope Database were analyzed by different prediction tools for B-cell, T-cell MHC class II and I. Then sequences aligned with the aid of ClustalW implemented in the BioEdit program.

Results and conclusions: For Bepipred test of B-cell the total number of conserved epitopes was 85. For Emini surface accessibility prediction, 36 conserved epitopes were passing the default threshold 1.0. In Kolaskar and Tongaonkar antigenicity, 36 conserved epitopes gave score above the default threshold 1.045. However, there are only three epitopes that pass the three tests (LYTIPEK, LAHQK, YPSVPS). The reference glycoprotein strain was analyzed using IEDB MHC-I binding prediction tool to predict T cell epitope. Twenty conserved peptides were predicted to interact with different MHC-I alleles. For MHC-II binding prediction there were 47 conserved epitopes found to interact with MHC-II alleles. The peptides GQILIPEMQ, FRRLSHFRK and FVGYVTTTF had the affinity to bind the highest number of MHC-II alleles. World population coverage for MHC-I most promising 3 peptides FVDLHMPDV, FVGYVTTTF and RLFDGTWVS was 67.42%, while the world population coverage for most promising MHC-II peptides was 99.77%, for the binding to MHC-I and MHC-II, The peptide FVG TTTF world population coverage was 99.31%.

Keywords: Immune; Epitope; Zoonotic; Tropical virus; Glycoprotein

Introduction

Rabies is one of the most fatal diseases caused by viral infection in humans [1]. There is no effective treatment after declaration of the infection, there is an effective management when applied as soon as possible after exposure, that's why most of the humans that develop symptoms of rabies virus infection inevitably die [1,2]. According to the World Health Organization, rabies is considered both a neglected zoonotic and tropical disease. Among all the *Lyssavirus* species known to exist today, Mokola virus is unique and appears to be exclusive to Africa. In contrast to all other known virus species in the genus *Lyssavirus* of the family *Rhabdoviridae*, its reservoir host has not been identified yet [3]. Nowadays Rabies is endemic in most parts of the world, and more effort is needed to develop affordable and effective vaccines to control or eliminate this disease [4]. Rhabdoviruses are single-stranded RNA viruses that possess non-segmented negative-sense genomes encoding five open reading frames and form enveloped, bullet-shaped virions [5].

Classical rabies vaccines consist of whole inactivated viruses that have the same antigenic characteristics as wild type viruses. Immunization with whole inactivated virus has been shown to induce virus-neutralizing antibodies directed against RVGP, activation of helper and cytotoxic T cells and protection against lethal intracerebral challenge with rabies virus [6,7]. The main reason that further research toward a new rabies vaccine candidate needed is the high cost of producing rabies vaccine in rabies-virus infected cell culture [8,9]. In some developing countries with high incidence of rabies, it is necessary to have a less expensive vaccine, allowing preventive immunization, preferentially after a single dose [10,11]. Other important reasons include the risks of production and administration of the current whole inactivated virus vaccine and the logistic concerns of a multi-vaccination schedule for pre and post-exposure vaccination [12,13]. The RVGP is the only antigen able to confer full protection against rabies and is the only component present in all new rabies vaccines that have been proposed [10]. The need for improved vaccines against virus infections has become an international priority [14]. On the other hand, there is a considerable difference in Individuals immune systems that in some cases individual's immune system will not respond adequately to

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protect against second exposure this is one of the reasons explaining the failure of immunization [15].

Nowadays computational techniques provide further information about viruses, a computational analysis study done by Badawi M, et al. on ZIKA virus, the envelope glycoprotein was obtained using protein database and the most immunogenic epitope for T and B cells involved in cell-mediated immunity were analyzed. They mainly focused on MHC class I potential peptides Using in silico analysis techniques [16]. In this study, the same techniques were used and we focused on both MHC class I and II with the world population coverage as well. The RNA-dependent RNA-polymerase together with phosphoprotein (P), functions as the transcriptase and replicase complex, the glycoprotein G is the only outer membrane protein responsible for virus entry and inducing protective immune responses [17]. In a previous study done by Ahmed et al. that aims to design a vaccine for Lagos Rabies virus using peptides of its glycoprotein G as an immunogenic part to stimulate protective immune response we proposed a very interesting T cell epitope (FVGYVTTTF) that have very strong binding affinity to both MHC1 and MHC11 alleles and it was found to bind 13 different alleles with world population coverage 97.3%, which indicates strong potential to formulate peptide vaccine for Lagos Rabies virus [18].

In this study, we aim to design an Epitope-Based Peptide Vaccine against Mokola Rabies virus using peptides of its glycoprotein G as an immunogenic part to stimulate a protective immune response, which is an extension of our previous studies on the other strains of Rabies virus.

Materials and Methods

Protein sequence retrieval

A total of 27 strains of rabies Mokola rabies virus strains' glycoprotein G were retrieved from NCBI (https://www.ncbi.nlm.nih.gov/protein). Database in November 2016. These 27 strains sequences retrieved are from different parts of the world (include 12 collected from South Africa, 6 from Zimbabwe, 2 from Nigeria, one from **Cameroon** and one from Central African Republic). Retrieved glycoprotein strains and their accession numbers and area of collection are listed in Table 1.

Determination of conserved regions

The retrieved sequences were then aligned to obtain conserved regions using multiple sequence alignment (MSA), sequences aligned with the aid of Clustal-W as Applied in the BioEdit program, version 7.0.9.1 (Hall, 1999) for finding the conserved regions among Rabies spike glycoprotein variants[19]. Later on, the candidate epitopes were analyzed by different prediction tools from Immune Epitope Database IEDB analysis resource (http://www.iedb.org/) [20].

B-cell Epitope Prediction

The reference sequence of glycoprotein G was subjected to different B cell tests [21].

Prediction of linear B-cell epitopes: Bepipred test from immune epitope database (http://tools.iedb.org/bcell/result/) [22] was used as linear B-cell epitopes prediction from the conserved region with a default threshold value of - 0.088.

Prediction of surface accessibility: By using Emini surface accessibility prediction tool of the immune epitope database (IEDB) (http://tools.iedb.org/bcell/result/) [23]. The surface accessible epitopes were predicted from the conserved region holding the default threshold value 1.0.

Accession Number	Date of collection	Country	Host
AMB61286.1	**2012**	**South Africa**	**Cat**
AMB61287.1	**2012**	**South Africa**	**Cat**
AMB61288.1	**2014**	**South Africa**	**Cat**
AGW00584.1	**2008**	**South Africa**	**AN**
ACV86802.1	**1993**	**Zimbabwe**	**Cat**
ACV86804.1	**1996**	**South Africa**	**Cat**
ACV86806.1	**1997**	**South Africa**	**Cat**
ADO15012.1	**NA**	**South Africa**	**Cat**
ADO15013.1	**NA**	**Zimbabwe**	**Canine**
ADO15015.1	**NA**	**Zimbabwe**	**Canine**
AGQ16856.1	1968	Nigeria	Shrew
AGQ16861.1	**1982**	**Zimbabwe**	**Cat**
AGQ16866.1	**1993**	**Zimbabwe**	**Cat**
AGQ16871.1	**1996**	**South Africa**	**Cat**
ACV86803.1	**1995**	**South Africa**	**Cat**
ACV86805.1	**1997**	**South Africa**	**Cat**
ADO15014.1	**NA**	**Zimbabwe**	**Canine**
AEE36617.1	**2006**	**South Africa**	**Cat**
ACV86801.1	**1998**	**South Africa**	**Cat**
ADQ01808.1	1968	Nigeria	Shrew
ABZ81205.1	**1974**	**Cameroon**	Shrew
ABZ81210.1	1981	Central African Republic	Rodent
P0C572.1	**AN**	**NA**	**NA**
*YP_142353.1	**NA**	**NA**	**NA**
AAA67271.1	**NA**	**NA**	**NA**
AAB26292.1	**NA**	**NA**	**NA**
AAB26296.1	**NA**	**NA**	**NA**

Table 1A: Virus Strains retrieved, their Accession numbers and area of collection: **Ref* sequence, NA: not available.

Prediction of Epitopes antigenicity: Using the Kolaskar and Tongaonkar antigenicity method to determine the antigenic sites with a default threshold value of 1.045. (http://tools.iedb.org/bcell/result/) [24].

MHC class I binding predictions

Analysis of peptide binding to MHC1 molecules was assessed by the IEDB MHC I prediction tool at http://tools.iedb.org/mhci, MHC-I peptide complex presentation to T lymphocytes undergo several steps. The attachment of cleaved peptides to MHC molecules step was predicted. Prediction methods were achieved by artificial neural network (ANN) method [25,26]. Prior to prediction, all epitope lengths were set as 9 mers, all internationally conserved epitopes that bind to alleles at score equal or less than 500 half-maximal inhibitory concentrations (IC50) were selected for further analysis [25].

MHC class II binding predictions

Analysis of peptide binding to MHC class II molecules was assessed by the IEDB MHC II prediction tool at (http://tools.iedb.org/mhcii/result/ [24,25]. For MHC-II binding predication, human allele references set were used. MHC class II groove has the ability to bind to peptides with different lengths. This variability in binding makes prediction as difficult as less accurate [26]. We used artificial neural networks that allows for simultaneous identification of the MHC class II binding core epitopes and binding affinity. All conserved epitopes that bind to many alleles at score equal or less than 1000 half-maximal inhibitory concentration (IC50) is selected for further analysis [27].

Population coverage calculation

All potential MHC I and MHC II binders from Mokola rabies virus glycoprotein G was assessed for population coverage against the whole world population with the selected MHC-I and MHC-II interacted alleles by the IEDB population coverage calculation tool at http://tools.iedb.org/tools/population/iedb_input [28].

Homology modeling

The reference sequence of Mokola rabies virus glycoprotein G was submitted to Raptor X on 21/12/2016, the 3D structure of glycoprotein was received on 22/12/2016 and then treated with chimera software to show the position of proposed peptides [29,30].

Results

B-cell epitope prediction

The reference sequence of Mokola rabies virus glycoprotein G was subjected to Bepipred linear epitope prediction, Emini surface accessibility and Kolaskar and Tongaonkar antigenicity methods in IEDB, to determine the binding to B cell, being in the surface and to test the immunogenicity, Table 1 and Figures 1-4.

Prediction of T helper cell epitopes and interaction with MHC I alleles

The reference glycoprotein G Mokola strain was analyzed using IEDB MHC-I binding prediction tool based on ANN-align with half-maximal inhibitory concentration (IC50) ≤ 500; the list of all epitopes and their correspondent binding MHC1 alleles were shown in (supplementary Table 1) while the list most promising epitopes that had Binding affinity with the Class I alleles along with their positions in the glycoprotein G of Mokola virus were shown in Table 2 and Figure 5.

Prediction of T helper cell epitopes and interaction with MHC II alleles

The reference glycoprotein (GP) strain was analyzed using IEDB MHC- II binding prediction tool based on NN-align with half-maximal inhibitory concentration (IC50) ≤ 1000; the list of all epitopes and their correspondent binding MHC11 alleles were shown in (supplementary

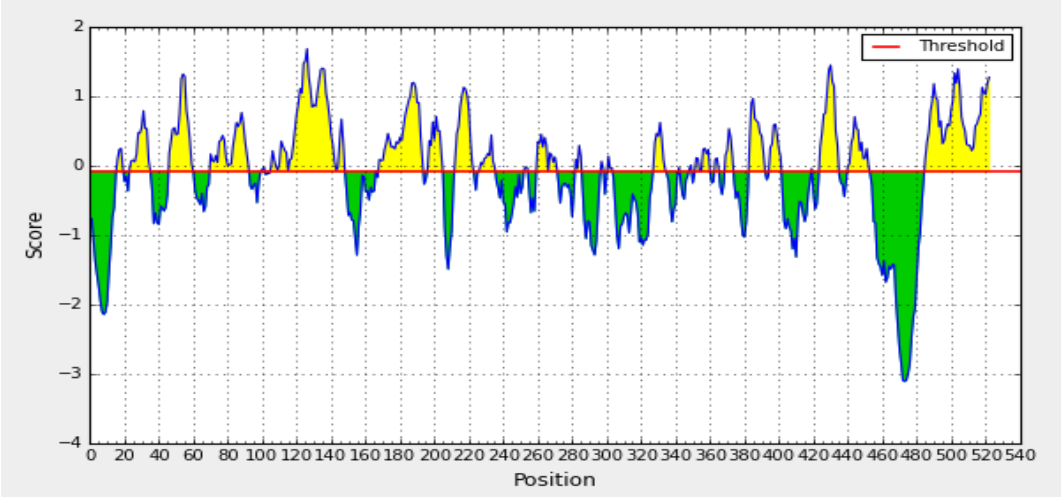

Figure 1: Present Bepipred Linear Epitope Prediction, the yellow space above threshold (red line) is proposed to be a part of B cell epitopes and the green space is not a part.

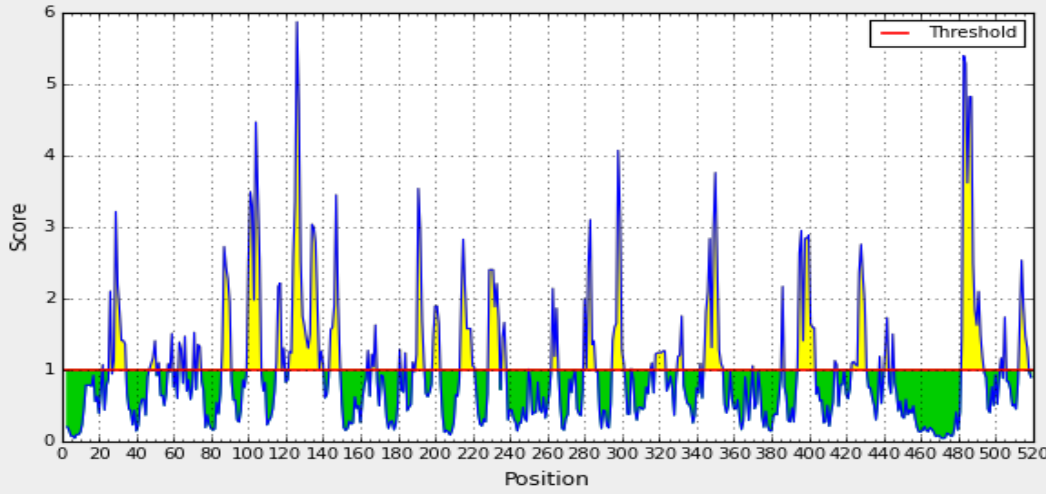

Figure 2: Present Emini surface accessibility prediction, the yellow space above threshold (red line) is proposed to be a part of B cell epitopes and the green space is not a part.

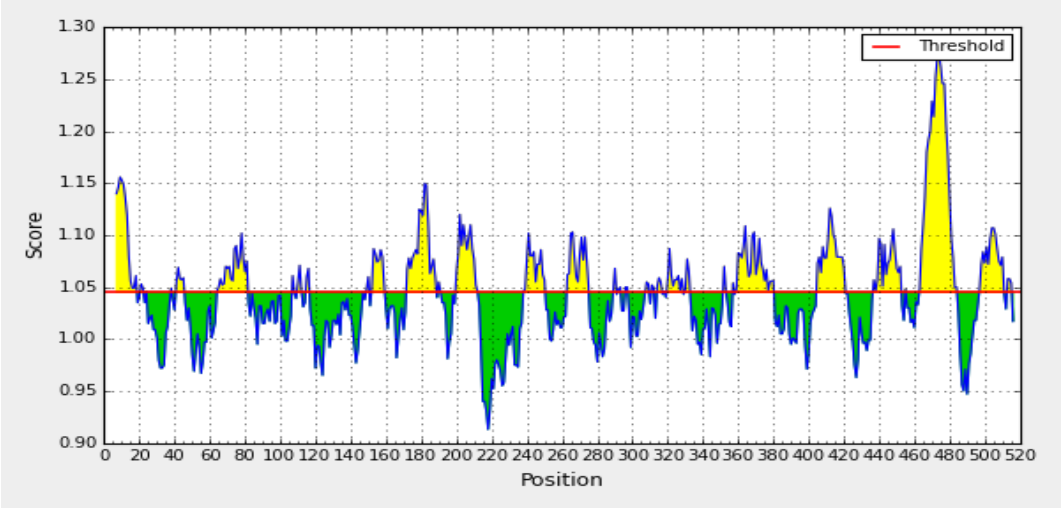

Figure 3: Present Kolaskar and Tongaonkar antigenicity prediction, Yellow areas above threshold (red line) are proposed to be a part of B cell epitope while green areas are not.

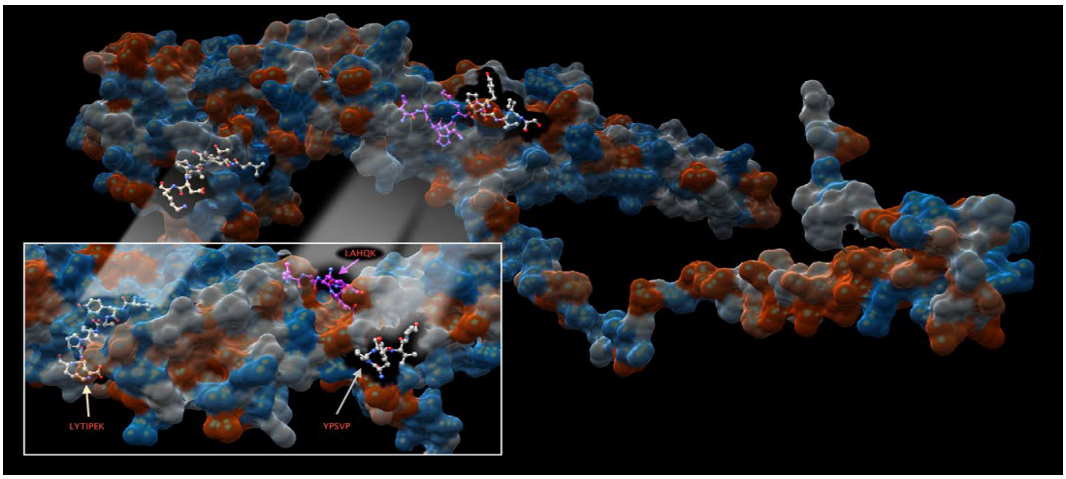

Figure 4: The Structural position of the most promising conserved B-cell epitopes of mokola virus glycoprotein G.

Peptide	Start	End	Length	Emini Surface Score TH=1.00	Kolaskar & Tongaonkar Score TH=1.045
LYTIPEKIEKWTP	23	35	13	2.045	1.009
*LYTIPEK	23	29	7	1.37	1.045
LAHQKV	70	75	6	0.721	1.125
*LAHQK	70	74	5	1.222	1.073
YPSVPSC	182	188	7	0.524	1.158
YPSVPS	182	187	6	1.235	1.116
*YPSVP	182	186	5	1.159	1.137

Table 1B: List of peptides with their surface accessibility score and antigenicity score.

Table 2) while the list most promising epitopes that had Binding affinity with the Class II alleles along with their positions in the glycoprotein G of Mokola rabies virus were shown in Table 3 and Figure 6.

Population coverage analysis

Population coverage test was performed to detect the world coverage of all epitopes binds to MHC1 alleles, MHC11 alleles and combined MHC 1 and MHC11 as well as selected most promising epitopes from each test.

Population coverage for MHC1 (Tables 4 and 5) and Figure 7

Population coverage for MHC11: The population coverage results of all peptides binding to MHC11 alleles along with their correspondent alleles were shown on Table 6 and the results of most promising three peptides were shown on Table 7.

Population coverage for both MHC1 and MHC11 alleles: This test was performed to the most promising epitope alone (FVGYVTTTF)

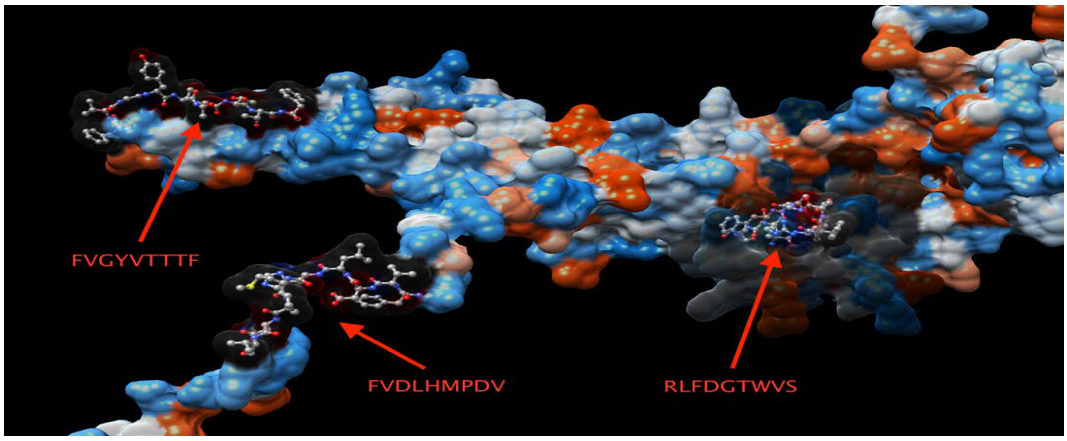

Figure 5: Structural Position of the three most promising conserved epitopes at glycoprotein G of Mokola rabies virus that interact with MHC I.

Epitope	Start	End	Allele	IC50	Percentile
FVDLHMPDV	434	442	HLA-A*02:01 HLA-A*02:06 HLA-C*05:01 HLA-C*05:01 HLA-C*08:02	42.44 8.38 60.39 60.39 236.93	0.5 0.1 0.4 0.4 0.1
FVGYVTTTF	93	101	HLA-A*23:01 HLA-A*32:01 HLA-B*15:01 HLA-B*35:01 HLA-B*53:01 HLA-B*58:01 HLA-C*03:03 HLA-C*05:01 HLA-B*53:01 HLA-B*58:01 HLA-C*03:03 HLA-C*05:01	337.71 115.41 99.41 18.33 206.88 494.71 247.75 251.83 206.88 494.71 247.75 251.83	0.4 0.3 0.2 0.2 0.7 0.5 1 0.7 0.7 0.5 1 0.7
RLFDGTWVS	253	261	HLA-A*02:01 HLA-A*02:06 HLA-A*32:01	39.12 79.54 119.28	0.5 0.7 0.4

***Refer to the conserved peptides that passed the Emini surface accessibility and Kolaskar and Tongaonkar antigenicity test.**

Table 2: List of most promising epitopes that had Binding affinity with MHC-I alleles along with their positions in the glycoprotein G of Mokola virus, IC50 and Percentile.

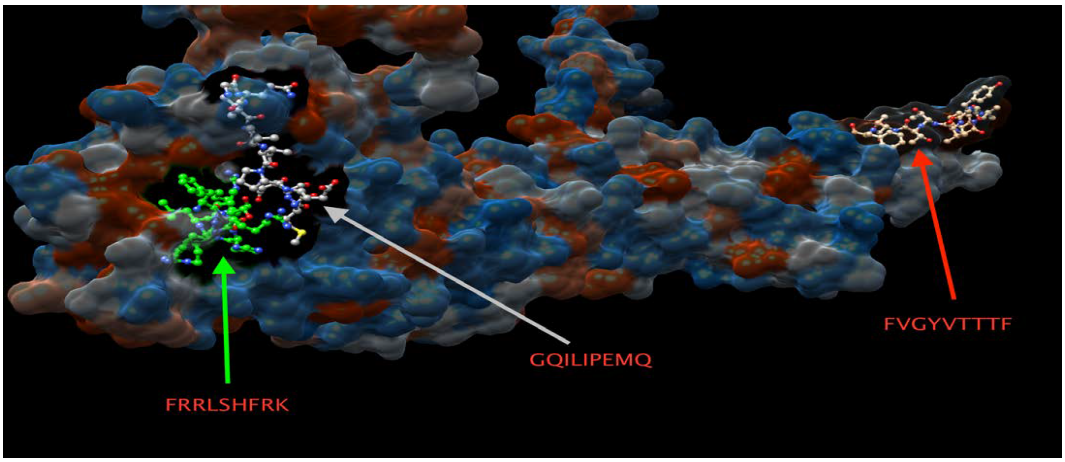

Figure 6: Molecular position of the most promising three epitopes of Mokola rabies virus that binds MHC II alleles.

Core Sequence	Alleles	Peptide Sequence	Start	End	IC50	Rank
FRRLSHFRK	HLA-DRB1*01:01	KPDVHVWCTPNQLIN	269	277	211.7	41.88
	HLA-DRB1*07:01	TKPDVHVWCTPNQLI	269	277	31.8	6.01
	HLA-DRB1*07:01	PDVHVWCTPNQLINI	269	277	45.7	8.06
	HLA-DRB1*07:01	VHVWCTPNQLINIHN	269	277	88.4	13.06
	HLA-DRB1*07:01	VWCTPNQLINIHNDR	269	277	230.3	22.99
	HLA-DRB1*08:02	DVHVWCTPNQLINIH	269	277	714.3	16.94
	HLA-DRB1*13:02	DVHVWCTPNQLINIH	269	277	199.2	9.8
	HLA-DRB1*13:02	KPDVHVWCTPNQLIN	269	277	288.3	12.41
	HLA-DRB1*13:02	HVWCTPNQLINIHND	269	277	413.6	15.53
	HLA-DRB3*01:01	DVHVWCTPNQLINIH	269	277	635.7	13.55
	HLA-DRB3*01:01	TKPDVHVWCTPNQLI	269	277	834.3	15.96
	HLA-DRB5*01:01	DVHVWCTPNQLINIH	269	277	245.4	24.28
	HLA-DRB5*01:01	PDVHVWCTPNQLINI	269	277	400.9	30.14
	HLA-DRB5*01:01	KPDVHVWCTPNQLIN	269	277	660.9	36.97
	HLA-DRB1*15:01	RAKVVSSWESYKGLP	509	517	114	11.1
	HLA-DRB1*15:01	AKVVSSWESYKGLPG	509	517	136.3	12.83
	HLA-DRB1*15:01	VPRAKVVSSWESYKG	509	517	205.5	17.26
	HLA-DRB5*01:01	RAKVVSSWESYKGLP	509	517	347.5	28.35
	HLA-DRB5*01:01	AKVVSSWESYKGLPG	509	517	641.3	36.53
	HLA-DRB5*01:01	PVPRAKVVSSWESYK	509	517	755.1	38.92
	HLA-DRB1*08:02	CTGVVNEAETYTNFV	83	91	693.4	16.47
	HLA-DRB1*08:02	TCTGVVNEAETYTNF	83	91	973.7	22
	HLA-DPA1*01/DPB1*04:01	VAFVVLVCLLRVCCK	470	478	393.4	12.94
	HLA-DPA1*01:03/DPB1*02:01	AFVVLVCLLRVCCKR	470	478	543.5	22.45
	HLA-DPA1*02:01/DPB1*01:01	TIVAFVVLVCLLRVC	470	478	368.5	26.01
	HLA-DPA1*02:01/DPB1*01:01	VAFVVLVCLLRVCCK	470	478	459.8	29.41
	HLA-DPA1*03:01/DPB1*04:02	VVLVCLLRVCCKRVR	470	478	110.6	10.34
FVGYVTTTF	HLA-DRB1*15:01	FRKLVPGYGKAYTIL	327	335	56.2	5.76
	HLA-DRB1*15:01	RKLVPGYGKAYTILN	327	335	64.2	6.6
	HLA-DRB1*15:01	LVPGYGKAYTILNGS	327	335	160.6	14.49
	HLA-DRB1*04:04	AHQKVPGFTCTGVVN	75	83	398.9	30.93
	HLA-DQA1*01:02/DQB1*06:02	CTGVVNEAETYTNFV	84	92	210.2	14.59
	HLA-DQA1*01:02/DQB1*06:02	TGVVNEAETYTNFVG	84	92	238.1	16.17
	HLA-DQA1*01:02/DQB1*06:02	GVVNEAETYTNFVGY	84	92	397.9	23.56
	HLA-DQA1*05:01/DQB1*03:01	CTGVVNEAETYTNFV	84	92	388.5	31.84
	HLA-DQA1*05:01/DQB1*03:01	GVVNEAETYTNFVGY	84	92	604.2	38.96
	HLA-DRB1*04:04	PVKGVLFNGIIKGPD	377	385	712.6	41.02
	HLA-DRB1*04:04	VKGVLFNGIIKGPDG	377	385	768.5	42.39
	HLA-DRB1*04:04	VLFNGIIKGPDGQIL	377	385	871.6	44.72
	HLA-DRB1*13:02	VKGVLFNGIIKGPDG	377	385	558.3	18.58
	HLA-DRB1*13:02	MEPVKGVLFNGIIKG	377	385	641.5	20.15
	HLA-DPA1*01/DPB1*04:01	QCMEPVKGVLFNGII	374	382	123.7	6.16
	HLA-DPA1*01/DPB1*04:01	MEPVKGVLFNGIIKG	374	382	176.9	7.88
	HLA-DPA1*01/DPB1*04:01	EPVKGVLFNGIIKGP	374	382	326.1	11.56
	HLA-DPA1*01:03/DPB1*02:01	QCMEPVKGVLFNGII	374	382	96.8	8.18
	HLA-DPA1*01:03/DPB1*02:01	CMEPVKGVLFNGIIK	374	382	115.9	9.18
	HLA-DPA1*01:03/DPB1*02:01	EPVKGVLFNGIIKGP	374	382	248.7	14.63
	HLA-DPA1*02:01/DPB1*01:01	QCMEPVKGVLFNGII	374	382	239.5	20.01
	HLA-DPA1*02:01/DPB1*01:01	QQCMEPVKGVLFNGI	374	382	307.1	23.38
	HLA-DPA1*02:01/DPB1*01:01	EPVKGVLFNGIIKGP	374	382	420.1	28
	HLA-DPA1*02:01/DPB1*01:01	VKGVLFNGIIKGPDG	374	382	628.4	34.49
	HLA-DPA1*03:01/DPB1*04:02	MEPVKGVLFNGIIKG	374	382	397.1	21.15
	HLA-DPA1*03:01/DPB1*04:02	EPVKGVLFNGIIKGP	374	382	506.4	23.76
	HLA-DPA1*03:01/DPB1*04:02	PVKGVLFNGIIKGPD	374	382	736.3	28.05
	HLA-DQA1*05:01/DQB1*03:01	EPVKGVLFNGIIKGP	374	382	466	34.64
	HLA-DQA1*05:01/DQB1*03:01	CMEPVKGVLFNGIIK	374	382	570.5	37.96
	HLA-DQA1*05:01/DQB1*03:01	QQCMEPVKGVLFNGI	374	382	992.2	47.84
GQILIPEMQ	HLA-DRB1*15:01	DADDFVDLHMPDVHK	435	443	815	36.73
	HLA-DQA1*05:01/DQB1*03:01	KSVSDVDLGLPHWGF	449	457	915	46.32
	HLA-DRB1*01:01	VSDVDLGLPHWGFWM	449	457	834	64.6
	HLA-DQA1*01:01/DQB1*05:01	NVYYKRVDKWADILP	351	359	817.6	12.91
	HLA-DRB1*09:01	SGVCSNVYPSVPSCE	177	185	823.5	34.86
	HLA-DRB1*13:02	GVCSNVYPSVPSCET	177	185	756.4	22.16

Table 3: List of most promising epitopes core sequence that had Binding affinity with MHC II alleles along with their positions in the glycoprotein G of Mokola rabies virus, IC50 and there Ranks.

and it was found to bind 15 alleles and gave population coverage of 99.31% as shown in below Figures 8 and 9.

Discussion

In this computational immunoinformatics study we suggest a new promising highly selective peptides vaccine against Mokola rabies virus for the first time according to our knowledge. We expect to obtain a peptide-based vaccine with high antigenicity and minimum allergic effect rather than the currently used vaccines. This challenge start after having good information about the protein structure of Mokola virus from literature review, then the reference sequence of Mokola rabies virus glycoprotein G was obtained from the NCBI. To determine the binding affinity of the conserves epitopes to B-cell and to examine the immunogenicity we

Epitope	Coverage
Epitope #2: ACKLTLCGR	5.36%
Epitope #3: AESSFTYFE	6.27%
Epitope #4: DEIEHLIVE	7.32%
Epitope #5: DIFTSSNGK	5.83%
Epitope #6: EAETYTNFV	2.50%
Epitope #7: ETNVYYKRV	2.50%
Epitope #8: ETYTNFVGY	35.79%
Epitope #9: FPLRHPLIS	8.42%
Epitope #10: FPLYTIPEK	10.84%
Epitope #11: FPSGVCSNV	2.53%
Epitope #12: FTKPDVHVW	10.31%
Epitope #13: FVDLHMPDV	47.68%
Epitope #14: FVGYVTTTF	40.63%
Epitope #15: GYGKAYTIL	21.38%
Epitope #16: GYVTTTFKR	5.36%
Epitope #17: HTPYPDSSW	3.90%
Epitope #18: IIKKREECL	10.55%
Epitope #19: IISPSIVEM	17.52%
Epitope #20: ILPSKGCLK	16.81%
Epitope #21: KESLLIISP	3.45%
Epitope #22: KLVPGYGKA	1.95%
Epitope #23: KQHMDLLKA	1.95%
Epitope #24: LAHQKVPGF	8.42%
Epitope #25: LFDGTWVSF	5.43%
Epitope #26: LIVEDIIKK	5.83%
Epitope #27: LVCDIFTSS	1.95%
Epitope #28: LVPGYGKAY	21.89%
Epitope #29: RICGFKDER	5.83%
Epitope #30: RLDEIEHLI	7.85%
Epitope #31: RLFDGTWVS	44.14%
Epitope #32: RRLSHFRKL	36.50%
Epitope #33: RSLKGACKL	4.41%
Epitope #34: SFPSAPVPR	5.36%
Epitope #35: SFRRLSHFR	9.14%
Epitope #36: TIVAFVVLV	2.50%
Epitope #37: TYFELKSGY	3.04%
Epitope #38: TYTNFVGYV	2.50%
Epitope #39: VFPLRHPLI	21.38%
Epitope #40: VGYVTTTFK	30.92%
Epitope #41: WKVSGDPRY	8.42%
Epitope #42: WTPIDMIHL	2.50%
Epitope #43: YEESLHTPY	27.67%
Epitope #44: YTILNGSLM	4.41%
Epitope #45: YTIPEKIEK	20.88%
Epitope #46: YTNFVGYVT	6.81%
Epitope #47: YVTTTFKRK	15.53%
Epitope set	**99.48%**

Table 4: Population coverage results for all epitopes binding to MHC1 alleles of Mokola rabies virus.

subjected the reference sequence of Mokola rabies virus glycoprotein G to IEDB database. Bepipred linear epitope prediction test, Emini surface accessibility test and Kolaskar and Tongaonkar antigenicity test were examined. For Bepipred test of B-cell the total number of conserved epitopes was 85. For Emini surface accessibility prediction, 36 conserved epitopes were passing the default threshold 1.0. In Kolaskar and Tongaonkar antigenicity, 36 conserved epitopes gave score above the default threshold 1.045. However, there are only three epitopes that pass the three tests (LYTIPEK, LAHQK, YPSVP). The reference glycoprotein strain was analyzed using IEDB MHC-I binding prediction tool to predict

Epitope	Coverage	Total HLA hits
Epitope #1: FVDLHMPDV	47.68%	**4**
Epitope #2: FVGYVTTTF	40.63%	**8**
Epitope #3: RLFDGTWVS	44.14%	**3**
Epitope set	**67.42%**	

Table 5: Population coverage of most promising 3 epitopes binds to MHC1 alleles of Mokola rabies virus

Epitope	Coverage
Epitope #1: ACRDAYNWK	11.21%
Epitope #2: ADDFVDLHM	28.50%
Epitope #3: CLKVGQQCM	16.59%
Epitope #4: DFVDLHMPD	27.73%
Epitope #5: DGQILIPEM	18.41%
Epitope #6: DYTLWLPED	28.16%
Epitope #7: EAETYTNFV	10.54%
Epitope #8: EKIEKWTPI	15.70%
Epitope #9: EMQSEQLKQ	18.23%
Epitope #10: EQLKQHMDL	6.40%
Epitope #11: FPLYTIPEK	21.87%
Epitope #12: FRKLVPGYG	55.84%
Epitope #13: FRRLSHFRK	98.13%
Epitope #14: FVDLHMPDV	83.26%
Epitope #15: FVGYVTTTF	99.31%
Epitope #16: GQILIPEMQ	83.56%
Epitope #17: GYVTTTFKR	23.19%
Epitope #18: HDYTLWLPE	67.74%
Epitope #19: HFRKLVPGY	23.90%
Epitope #20: HVWCTPNQL	43.67%
Epitope set	**99.93%**

Table 6: Population coverage results for all epitopes binding to MHC11 alleles of Mokola rabies virus.

Epitope	Coverage	Total HALA Hits
Epitope #1: FRRLSHFRK	98.13%	**15**
Epitope #2: FVGYVTTTF	99.31%	**15**
Epitope #3: GQILIPEMQ	83.56%	**8**
Epitope set	**99.77%**	

Table 7: The most promising three epitopes that binds to MHC11 along with their coverage and total HALA hits

T cell epitope. Twenty conserved peptides were predicted to interact with different MHC-I alleles. For MHC-II binding prediction there were 47 conserved epitopes found to interact with MHC-II alleles. The peptides GQILIPEMQ, FRRLSHFRK and FVGYVTTTF had the affinity to bind the highest number of MHC-II alleles. World Population coverage for MHC-I most promising 3 peptides FVDLHMPDV, FVGYVTTTF and RLFDGTWVS was 67.42%, while the world population coverage for most promising MHC-II peptides was 99.77%, for the binding affinity to MHC-I and MHC-II the peptide FVGYVTTTF was found to bind 15 different alleles and gave population coverage of 99.31%. This finding shows a very strong potential to formulate an epitopes-based peptide vaccine for Mokola Rabies virus. Interestingly the same peptide FVGYVTTTF was also proposed as epitopes-based peptide vaccine for Lagos rabies virus in a previous study done by Ahmed et al. that aims to design a vaccine for Lagos Rabies virus. The binding affinity to both MHC1 and MHC11 alleles was found to be 13 different alleles with world population coverage 97.3% [18]. According to these interesting findings, a very promising vaccine for both Mokola and Lagos rabies virus can be formulated. This could lead to a universal epitopes-based peptide vaccine for all rabies virus strains.

MHC Class	Coverage [a]	Average hit [b]	PC90 [c]
Class I	67.42%	1.48	0.31

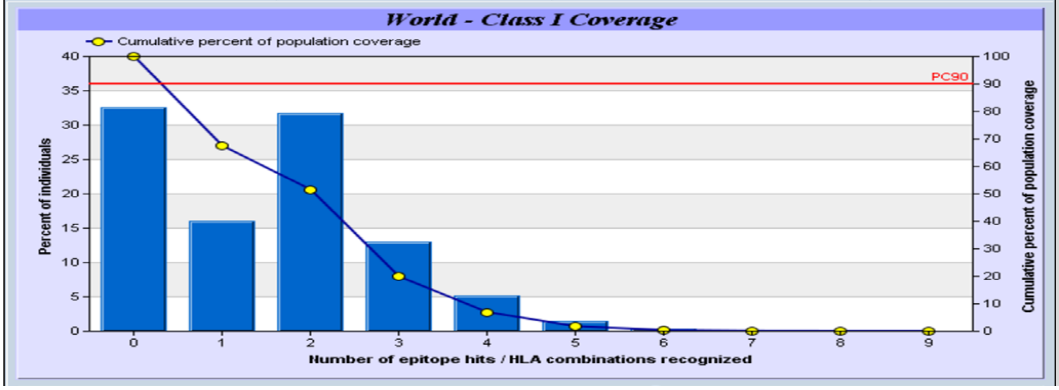

Figure 7: Population coverage of most promising 3 epitopes binds to MHC1 alleles of Mokola rabies virus.

MHC Class	Coverage [a]	Average hit [b]	PC90 [c]
Class I and II	99.31%	3.24	2.12

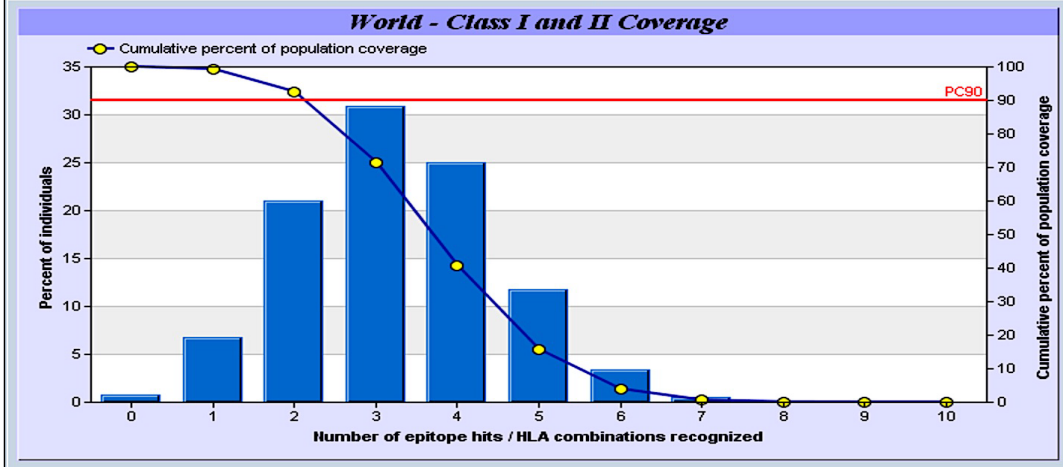

Figure 8: Molecular position of the most promising epitope for population coverage that binds both MHC1 and MHC11 alleles.

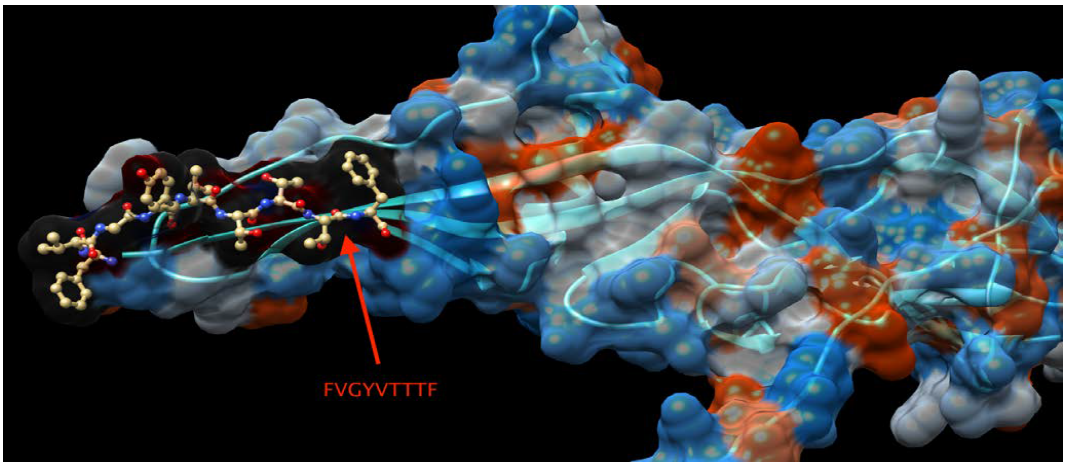

Figure 9: Molecular position of the most promising epitope for population coverage that binds both MHC1 and MHC11 alleles.

Conclusion

To our knowledge this study was the first one that propose epitopes-based peptide vaccine for Mokola rabies virus, which is expected to be highly antigenic with a minimum allergic effect than the currently used biochemical vaccines. Farther more this study proposed a promising peptide FVGYVTTTF that shown a very strong binding affinity to both MHC1 and MHC11 alleles, it was found to bind 15 different alleles with world population coverage of 99.31%, which indicates a strong potential to formulate peptide vaccine for Mokola Rabies virus.

Recommendations

An *in vivo* evaluation of the most promising peptides in this study is recommended that we expect to give a high impact of this research especially for peptide FVGYVTTTF that could be a promising epitopes-based peptide vaccine for multiple strains.

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